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RESEARCH ARTICLE

Carbohydrate and protein are an attribute to enhance the life-history determinants in *Drosophila*Ramesh B.Y.¹ Neethu B. K.² and *Harini B. P.²

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Abstract

Dietary restriction extends life span across a vast diversity of taxa, but the key nutritional components driving this process and how they interact remain uncertain. In *Drosophila*, while a substantial body of research suggests that protein is the major dietary component affecting longevity, recent studies claim that carbohydrates also play a central role. To clarify how nutritional factors influence longevity, nutrient consumption and lifespan were measured on a series of diets with varying casein and sugar content. Increased dietary carbohydrate or protein concentration does not always result in increased longevity. Our study indicates that the combination of carbohydrate and protein has certainly experiences significant effects with increased values rather than only carbohydrates nor only protein for the life history traits recorded. Thus the media enriched with the rich sources of food composition as resulted with enhanced mating activity, productivity and longevity in *Drosophila melanogaster*.

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Introduction

During life, body tissues constantly require a specific quantity and proportion of nutrients in order to attain optimal growth and performance (Bauerfiend and Fisher, 2005). Deficiency or imbalance of fat, carbohydrate or protein can affect characters such as somatic growth and reproduction. *Drosophila* has proved a useful model organism for studies of the mechanisms of dietary restriction (DR) (Tatar 2012 and Partridge *et al.*, 2011). Carbohydrates are important dietary components for many omnivorous and herbivorous animals, including both humans and livestock. Carbohydrates provide energy for many reactions and processes flowing inside cells. Most organisms can tightly adjust their metabolism according to the availability of dietary components, including carbohydrates. Physiological effects of carbohydrates depend on their type and dosage, as well as on the physiological state of an organism (Wheeler and Pi-sunver., 2008). Very low carbohydrate intake restricts an organism's available energy and may slow down growth and regeneration, thereby altering survival and health. However, low carbohydrate intake has been proposed as a possible intervention to decrease the risk of, and complications related to, metabolic diseases such as obesity and metabolic syndrome (Giugliano *et al.*, 2008).

Nutritional environment is a potent mediator of an organism lifespan, in particular dietary restriction has been constantly found to extend lifespan across a vast range of animal taxa including Yeast (Lin *et al.*, 2002), Fruit flies (Chippidale *et al.*, 199), Mice (Weindruch and walford, 1982), Rhesus monkey (Roth *et al.*, 1999). The influence of distinct carbohydrates on ageing has previously been tested for several different model organisms, including the fruit fly *Drosophila melanogaster* (Diptera: Drosophilidae). One of the pioneering studies in this field was performed by Hassett (Cho *et al.*, 2011). However, in Hassett's experiment, flies fed glucose solution had a slightly shorter life span than those on sucrose. In several studies has been demonstrated that increased intake of protein may increase protein synthesis, decrease protein breakdown, reduce fat accumulation, and increase fat-free mass (Kerksick *et al.*, 2006; Piatti *et al.*, 1994) has been demonstrated. Therefore protein supplementation or a high-protein diet (HPD) is recommended to build the muscle in athletes, to prevent muscle wasting in severe illness, and

to lose the fat in treatment of obesity. The most popular forms of protein supplements are milk proteins, whey and casein. Casein, which makes up approximately 80% of the milk protein, is considered “slow” protein because, in comparison with whey protein, is emptied from stomach more slowly and amino acids from casein appear in the blood more slowly, and the response lasts longer. It is believed that while whey protein affects protein balance mostly by stimulation of protein synthesis, casein works to decrease protein breakdown (Boirie *et al.*, 1997).

Casein contains high proportions of all essential amino acids and high amounts of glutamine and proline but, in comparison with blood meal, provides relatively low amounts of glycine and cysteine (Li *et al.*, 2011). Therefore, it may be suggested that chronic intake of high amounts of casein may induce the imbalance in amino acid concentration in body fluids. This may affect a number of biochemical pathways, susceptibility to oxidative damage, and the response of the body to different physiological and pathological conditions, such as starvation or illness. There is scarce information available on how nutrition affects life history traits in *Drosophila*. The importance of diet is often underestimated in experimental design (Prasad *et al.*, 2003). Hence, there is a growing need to investigate diet related effects behind variations in traits of importance for fitness. The physiological changes intern affects life history and fitness traits such as fecundity and reproduction. Many organisms live in variable environments, which pose substantial challenges to survival and reproduction. Dietary components may act independently of their role in nutrition to modulate intracellular signaling pathways directly.

In *Drosophila* the impact of dietary yeast on longevity is dependent on the target of rapamycin (TOR) signaling pathway (Kapahi *et al.*, 2004). Much of work that characterizes the myriad affects of diet in invertebrate system is proving relevant to mammalian aging and physiology. The combination of taste, smell, texture appearance influences and aversions may link with the nutritional value of the perceived food (Goff and Klee, 2006). However, the effect of carbohydrate diets, and particularly the type of carbohydrate, as well as the protein-to-carbohydrate ratio on reproduction and life span are poorly investigated and generally studied in comparatively simple organisms like *Drosophila melanogaster*, which is intensively used as a model for nutritional studies. Over the last decade, several studies explored the effect of diet on life span, reproduction, behavior, and adaptation of fruit flies (Lee *et al.*, 2008; Vigne and Frelin, 2010).

In light of the above information the present study is to understand the effect of variable diet composition in combinations of sucrose and casein on life history traits and longevity in systematic approach to evaluate on the basis of mating propensity, fecundity, fertility and longevity.

Materials and methods

Fly stocks

Drosophila melanogaster (Oregon K) stock was obtained from *Drosophila* stock center, University of Mysore, Mysore, India. The fly stocks were routinely cultured in standard wheat cream agar medium. From this stock about 200-250 eggs were collected and placed in culture bottles (about 10 to 50 eggs/bottle). The newly hatched flies from these stocks were considered to be the parental stock. About 30 males and females were separated by gender and were transferred to the fresh media vials containing variable diet composition of casein, sucrose and sucrose plus casein and were aged for 2days. On the 3rd day of eclosion an unmated male and a virgin female was pair mated. Single pair mated flies were screened for mating propensity (courtship and copulation duration), fecundity, fertility and longevity.

Different doses of carbohydrate (sucrose) and protein (casein), (casein was dissolved in 0.1N NaOH) as a source of nutrients (were procured from MP Biomedicals, Bangalore), propionic acid as mold inhibitor and soji and agar for standard culture is being used and the appropriate concentration of nutritional composition is as follows (Table 1).

Assessment of mating propensity

Mating propensity was recorded accordingly with slight modification (Tanuja *et al.*, 2001; Bacigalupe *et al.*, 2007). Single pair mating was allowed to mate in an empty vial to record the duration of courtship and copulation. The time taken by male to mount on female (courtship duration) and the time from mounting to detaching (copulation) that is mating activity were observed and record for 60min. the pairing of flies from the time of mounting to detaching was recorded. The pairs which do not mate within a stipulated time of 60 minutes were discarded.

Assessment of fecundity

The life time fecundity is defined as the number of eggs laid by an individual during its lifetime (Birch *et al.*, 1963). For the assessment of lifetime fecundity, the method of Buck *et al.*, 1993 was followed. The same set of flies was used to observed for mating propensity were used to assess the fecundity, mated males from each pair was isolated and monitored for longevity, while females were transferred into separate vials containing variable concentration of carbohydrate and protein medium. Fecundity was recorded by counting the number of eggs laid by the mated female. Likewise, each replicate was transferred to the next set of fresh food vials containing medium

every alternate day and about six successive changes were made. Immediately after each transfer the vials were checked for the eggs laid and were counted under stereomicroscope.

Assessment of fertility

The fertility was assessed according to protocol of Singh, 1997. The same set of flies used to assess fecundity were continued to assess fertility (total number of adults emerged). Further, the number of flies that emerged from all the experimental trails for each of diet concentration was recorded.

Assessment of longevity

The longevity of the same set of parental flies (both males and females) used for fecundity and fertility were recorded for longevity from the day of emergence to mortality. Longevity was assessed using the modified protocol of Luckinbill and Clare, 1985.

Statistical analysis

One-way ANOVA was performed for the said life history parameters. Multiple comparison were made using Turkey's HSD test at probability level $P < 0.05$. The analysis was performed using the statistical presentation system software package (SPSS Inc 2008) 17.0 for MS Windows.

Results

Observation of mating propensity

Table 2 reveals the mean courtship and copulation duration of *D.melanogaster* fed with variable diet composition of sucrose and casein along with control. The mean courtship duration has prolonged in the sucrose fed flies than control but the differences are insignificant in lower and mid concentrations of sucrose while it was significant with control in high concentration of sucrose. In casein and sucrose plus casein fed flies the courtship duration was lesser than control and the differences were significant between control for all the three concentrations of casein and sucrose plus casein diet.

The mean copulation duration of flies fed with sucrose plus casein diet has prolonged than control significantly, while in sucrose source copulation duration was decreased significantly than control. Similar to courtship duration, copulation duration has also significantly increased than control in all diet a source that is sucrose, casein and sucrose plus casein at higher diet concentration. While it lesser in low concentration of casein followed by the mid concentration of casein. Drastic increase in copulating time has been recorded in sucrose plus casein diet rather than casein or sucrose diet.

Thus the study reveals that higher the concentration of combination of carbohydrate and protein experiences significant effect on mating propensity rather than only carbohydrate nor protein in the diet. In addition to this the flies fed only with casein perform better when compared to flies fed only with sucrose diet. Thereby both carbohydrate and protein together were the important components which necessitate the mating activity of the flies and also casein has provided increased intensity of mating activity than with only sucrose.

Assessment fecundity and fertility

The mean fecundity and fertility of *D.melanogaster* on exposure to variable sources of diet fed in different concentrations has been depicted in Table 3. The data reveals significant increase in the mean number of eggs and as well adult emergence in the variable sources of food diets of protein as well as both sucrose and casein than control diet, but the flies fed only with sucrose diet has shown reduced fecundity and adult emergence (fertility) than control. The comparison between the different concentrations of diets within sources of diet has shown that the higher the concentration (high concentration of sucrose, high concentration of casein and high concentration of sucrose plus casein) has led to drastic increase in the fecundity and fertility followed by mid and low concentrations. Thus from the data it was evident that the diet enriched with both carbohydrate and protein composition leads to better productivity rather than only carbohydrate nor protein.

Assessment of longevity

Table 4 shows the mean longevity of males and females of *D.melanogaster* flies fed with variable sources of diet in different concentration. The mean lifespan of females were significantly greater than males in all the concentrations of variable diet sources and also in control. Sucrose and sucrose plus casein fed diet have shown increased longevity than control except the casein fed source. Sucrose plus casein fed diet have shown increased longevity significantly than control. While the flies fed with casein diet has shown decreased longevity. The mean lifespan has decreased in casein fed flies than control as well as sucrose and sucrose plus casein which means that high amounts of only protein intake causes imbalances in the lifespan with reduced longevity.

Table 1. Enriched food media administered to *D. melanogaster* flies

Diet→ Components↓	Control media	Sucrose			Casein			Sucrose+Casein		
		LD	MD	HD	LD	MD	HD	LD	MD	HD
Water	1000ml	1000ml	1000ml	1000ml	1000ml	1000ml	1000ml	1000ml	1000ml	1000ml
Agar	10g	10g	10g	10g	10g	10g	10g	10g	10g	10g
Soji	100g	100g	100g	100g	100g	100g	100g	100g	100g	100g
Sucrose	-----	20g	40g	60g	-----	-----	-----	20g	40g	60g
Casein	-----	-----	-----	-----	2.5g	5g	7.5g	2.5g	5g	7.5g
Propionic acid	7.5ml	7.5ml	7.5ml	7.5ml	7.5ml	7.5ml	7.5ml	7.5ml	7.5ml	7.5ml
Jaggery	100g	100g	100g	100g	100g	100g	100g	100g	100g	100g

LD= Low Diet concentration; MD= Mid Diet concentration; HD= High Diet concentration; g= grams; ml= mille liter

Table 2. Mean (\pm SE) Mating propensity of *Drosophila melanogaster* on exposure to variable concentration of carbohydrate and protein

Diet→ Traits→ Concentrations↓	N	Sucrose	Casein	Sucrose plus casein	Sucrose	Casein	Sucrose plus casein
		Courtship duration	Courtship duration	Courtship duration	Copulation time	Copulation time	Copulation time
Control	30	5.70 \pm 0.23 a	5.70 \pm 0.23 a	5.70 \pm 0.23 a	16.17 \pm 0.24 a	16.17 \pm 0.24 a	16.17 \pm 0.24 a
LD	30	5.76 \pm 0.25 a	2.80 \pm 0.18 b	1.60 \pm 0.14 b	12.10 \pm 0.29 b	18.23 \pm 0.24 b	17.20 \pm 0.24 a
MD	30	5.80 \pm 0.25 a	3.13 \pm 0.16 b	2.13 \pm 0.13 b	14.10 \pm 0.26 c	19.20 \pm 0.18 c	20.61 \pm 0.41 b
HD	30	6.79 \pm 0.22 b	4.08 \pm 0.15 c	4.19 \pm 0.26 c	15.06 \pm 0.23 d	25.20 \pm 0.13 d	28.41 \pm 0.28 c
ANOVA		F=4.629 P<0.05	F=48.550 P<0.05	F=87.702 P<0.05	F=44.030 P<0.05	F=60.910 P<0.05	F=38.818 P<0.05

Note: Means in each column followed by different alphabetical letter with in the same life stage were significantly different by Tukey HSD (P<0.05); N= number of individuals.

Table 3. Mean (\pm SE) Viability of *Drosophila melanogaster* on exposure to variable concentration of carbohydrate and protein

Diet→ Traits→ Concentrations↓	N	Sucrose	Casein	Sucrose plus casein	Sucrose	Casein	Sucrose plus casein
		Fecundity	Fecundity	Fecundity	Fertility	Fertility	Fertility
Control	30	114.0 \pm 2.43 a	114.0 \pm 2.43 a	114.0 \pm 2.43 a	100.0 \pm 1.53 a	100.0 \pm 1.53 a	100.0 \pm 1.53 a
LD	30	97.01 \pm 2.0 b	127.4 \pm 1.89 b	144 \pm 2.11 b	87.42 \pm 1.19 b	119.30 \pm 1.22 b	120 \pm 1.25 b
MD	30	102.0 \pm 1.66 b	145.0 \pm 2.19 c	152 \pm 1.31 c	91.71 \pm 1.43 bc	130 \pm 1.61 c	134 \pm 1.56 c

HD	30	119±2.96 a	149.56±1.39 c	163±2.29 d	94.63±0.81 c	134.23±1.67 c	156±2.33 c
ANOVA		F=19.436 P<0.05	F=49.588 P<0.05	F=11.481 P<0.05	F=17.15 P<0.05	F=100.68 P<0.05	F=18.142 P<0.05

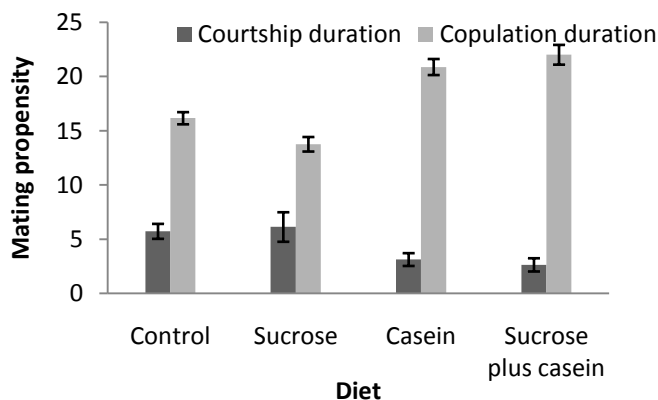
Note: Means in each column followed by different alphabetical letter with in the same life stage were significantly different by Tukey HSD (P<0.05); N= number of individuals

Table 4. Mean (±SE) longevity of *Drosophila melanogaster* on exposure to variable concentration of carbohydrate and protein

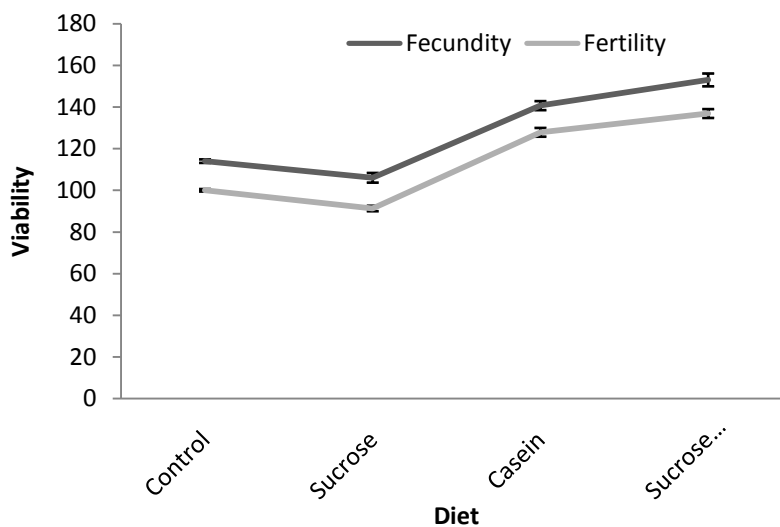
Diet→ Traits→ Concentrations↓	N	Sucrose Male	Casein Male	Sucrose plus casein Male	Sucrose Female	Casein Female	Sucrose plus casein Female
Control	30	33.16±0.69 a	33.16±0.69 ab	33.16±0.69 a	36.30±0.90 a	36.30±0.90 ab	36.30±0.90 a
LD	30	36.23±0.86 a	30.13±0.88 a	49.79±1.02 b	37.62±0.98 a	34.26±0.74 a	52.41±1.60 b
MD	30	46.06±1.26 b	34.61±0.81 b	55.92±0.87 c	49.23±1.33 b	37.81±0.85 b	57.39±0.90 c
HD	30	50.10±1.10 c	35.52±0.91 b	56.0±1.02 c	53.21±1.13 b	38.63±1.01 b	59.33±1.28 c
ANOVA		F=63.038 P<0.05	F=8.062 P<0.05	F=18.496 P<0.05	F=58.481 P<0.05	F=4.709 P<0.05	F=74.638 P<0.05

Note: Means in each column followed by different alphabetical letter with in the same life stage were significantly different by Tukey HSD (P<0.05); N= number of individuals.

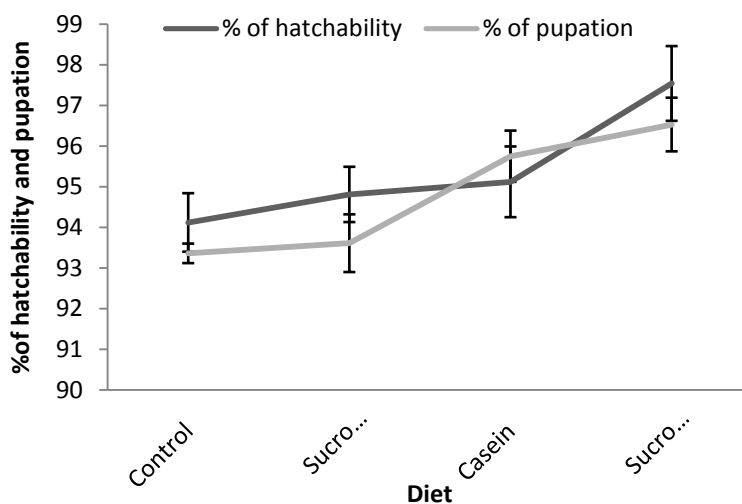
Graph 1. Mean (±SE) mating propensity of *Drosophila melanogaster* on exposure to Sucrose, casein and Sucrose plus casein



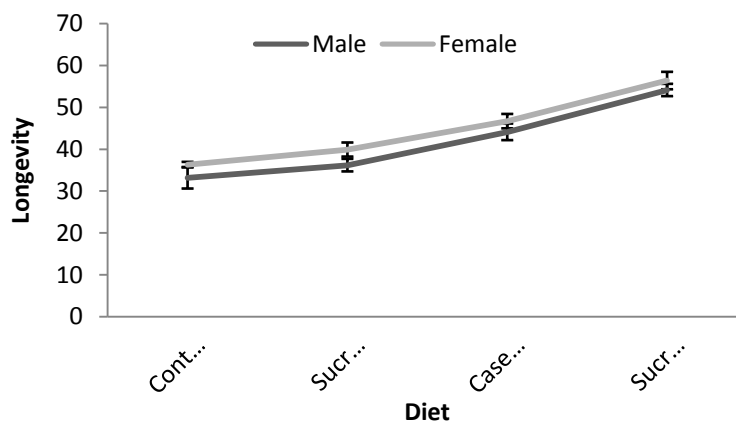
Graph 2. Mean (±SE) Viability of *Drosophila melanogaster* on exposure to Sucrose, Casein and sucrose plus casein



Graph 3. Mean (\pm SE) % of hatchability and pupation of *Drosophila melanogaster* on exposure to Sucrose, Casein and sucrose plus casein



Graph 4. Mean (\pm SE) Longevity of *Drosophila melanogaster* on exposure to Sucrose, Casein and sucrose plus casein



Discussion

In most invertebrate systems, dietary restriction is applied somewhat nontraditionally in that food quality, rather than quantity, is manipulated through dilution of the nutritional components in the medium (Pletcher *et al.*, 2005). This is in contrast to most rodent studies, where a fixed diet is provided to animals individually, and all of the food is consumed (Weindruch and Walford, 1988). Moreover, separate labs often employ divergent diet-restriction protocols involving different levels of nutrient dilution and alteration of dietary components. Fitness is multifaceted thing and the relative contributions of different traits to fitness vary in different environments and contexts. Life history evolution and population dynamics are fundamentally linked because formal life-history theory developed out of models of population growth in age-related populations (Cole, 1954; Gadgil and Bossert, 1970; Cholesworth, 1994), and moreover, life history traits like survivorship and fecundity and their sensitivity to density, are the major determinants of population dynamics (Mueller *et al.*, 2000). The increase in copulation duration as resulted in increase productivity which opines with the results of Harini, 2011, while mating activity has pronounced in protein fed diet than neither control nor carbohydrate (fig 1). Thus protein is very essential attribute for the mating propensity.

In addition this fecundity and fertility reduced in sucrose enriched medium than casein (fig 2). This is in accordance with earlier studies for the flies reared on high sucrose media (Wang and Clark, 1995; Bownes and Blair, 1986). Females showed relatively low egg-laying capability in carbohydrate diet, while egg production in protein fed females was significantly higher than carbohydrate fed females and are not surprising as studies on *Drosophila* and other insects have found similar results (Mattson, 1980; Cook, 1995; Markow *et al.*, 2001; Jervis and Boggs, 2005; Nestel and Nemny-Lavy, 2008). Therefore reproductive potentiality could not be determined by only carbohydrate or protein. The diet containing a mixture of carbohydrate and protein are required to maximize the reproduction and as well as fertility.

The females have shown increased lifespan than males. Protein diet (casein) have shown decreased lifespan than control and only carbohydrate (sucrose) fed flies, but the combination of both carbohydrate and proteins plays very important role in enhancing the life span, which is contradicting the Mair *et al.*, 2005. In invertebrates, the ratio between protein and non-protein intake, rather than calories, is fundamental to the relationship between diet and longevity (Simpson and Raubenheimer, 2009). Recent studies in organisms including *Drosophila melanogaster*, Queensland fruit fly (*Bactrocera tryoni*), the Tephritid fruit fly (*Anastrepha ludens*), and the field cricket (*Teleogryllus commodus*) increasingly support the view that consumption of an optimal ratio of carbohydrate to protein, with or without changes in caloric intake, is the key determinant of lifespan (Carey *et al.*, 2008; Fanson *et al.*, 2009; Lee *et al.*, 2008; Maklakov *et al.*, 2009). The present study shows that a relatively high C: P (8:1) maximizes longevity in Female (Fig.4), consistent with previous findings on females reporting an optimal C: P of 16:1 (Lee *et al.*, 2008). Our results indicate that high protein consumption limits longevity, as previously suggested Mair *et al.*, (2005). Thus mating activity, life history traits and longevity are controlled by the interplay between carbohydrate and protein intake, in sharp contrast to claim that carbohydrates and proteins have an impact on DR-mediated of the mating propensity, productivity and longevity.

Thus we emphasize the importance of carbohydrate and protein intake for optimal life history and lifespan. Thus from the study it is evident that the combination of carbohydrate and protein has certainly experiences significant effects with increased values rather than only carbohydrates nor only protein for the life history traits recorded. Thus the media enriched with the rich sources of food composition as resulted with enhanced mating activity, productivity and longevity in *D.melanogaster*. Thus the perceptions of diet sources are variable in the different argue and hence the fruit flies also response variably to distinct nutritional composition that is between carbohydrate and protein.

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